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14. ABSTRACT
Status:
Understand cell-directed assembly and use it to direct the formation of new bio/nano interfaces and unique cellular behaviors
-Investigated the inclusion of multiple amphipathic components to control and tailor interfacial structures and functions
-Created new interfaces by incorporating non-native functional proteins to yield new functionalities
Extend cell-directed assembly to immobilize various cell types
-Encapsulated several new cell lines, including mammalian cells, in nano-structured hosts; investigated the evolving nano-structure and bio/nano interface with grazing incidence small angle x-ray scattering along with epifluoresecence and confocal scanning laster
-Used properties of nano-structure to incorporate nutrients and growth vital to different cell types in order to extend cell assembly

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			BRINKER, JEFFREY
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1. Biocompatible and Biomimetic Self-Assembly of Functional Nanostructures

The Air Force Office of Scientific Research Project Number FA9550-04-1-0087

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FY07 ~~Annual Report~~
Final Report

2. Objectives - Immobilization of individual cells and collections of cells in well-defined, reproducible, nano-to-microscale structures that allow structural and functional manipulation and interrogation is important for developing new classes of biotic/abiotic materials, for establishing the relationship between genotype and phenotype, and for elucidating responses to disease, injury/stress, or therapy – primary goals of biomedical research. Although there has been considerable recent progress in investigating the response of cells to chemical or topological patterns defined lithographically on two-dimensional (2D) surfaces, it is time to advance from 2D adhesion on dishes/fluidic devices to 3D architectures that better represent the natural nanoporous and 3D extracellular matrix (ECM). 3D immobilization in nanostructured hosts enables cells to be surrounded by other cells, maintains fluidic connectivity/accessibility, and allows development of 3D molecular or chemical gradients that provide an instructive background to guide cellular behavior. Although 3D cell immobilization in polymers, hydrogels, and inorganic gels has been practiced for decades, these approaches do not provide for bio/nano interfaces with 3D spatial control of topology and composition important to both the maintenance of natural cellular behavior patterns and the development of new non-native behaviors and functions. In particular for ALL previously reported approaches there was no apparent effect of the cell on the surrounding host nor any apparent means to purposefully use the nanostructured host to develop new cellular behaviors. Here we show that cell directed assembly (CDA) to be a unique distinguishing approach to prepare new bio/nano interfaces and to develop new cellular behaviors.

3. Status of Effort – During the past year we have extended the CDA approach in three significant areas: 1) incorporation of multiple amphipathic components; 2) inclusion of new cell lines; and 3) spatially defined cellular integration via a new lithography with life approach. Objectives and results for FY07 are summarized as follows:

- Understand cell-directed assembly and use it to direct the formation of new bio/nano interfaces and unique cellular behaviors
  - Investigated the inclusion of multiple amphipathic components to control and tailor interfacial structures and functions
  - Created new interfaces by incorporating non-native functional proteins to yield new functionalities

- Extend cell-directed assembly to immobilize various cell types
  - Encapsulated several new cell lines, including mammalian cells, in nanostructured hosts; investigated the evolving nanostructure and bio/nano interface with grazing incidence small angle x-ray scattering (GISAXS) along with epifluoresecence and confocal scanning laser microscopy.
  - Used the properties of the nanostructure to incorporate essential nutrients and growth and transcription factors vital to different cell types in order to extend cell-directed assembly to mammalian cells

- Pattern cells to create functional multi-cellular materials where nanostructure is used to influence cell-to-cell communication and thereby cellular behavior
  - Employed selective wetting to pattern living cells into functional and connected arrays
  - Demonstrated a potentially new pathway of cellular communication among individual, nanoconfined cells

4. Accomplishments and New Discoveries

4.1. Understand, develop, and control the characteristics of cell-directed assembly

4.1.1. Inclusion of multiple amphipathic components to control and tailor interfacial structure and function

Cellular plasma membranes incorporate multiple amphipathic components, including phospho- and glycolipids, cholesterol, and integral and peripheral proteins. Our previous work has focused on using single, short chained lipid species as (nano)structure directing agents, where the size and shape of the lipids establish the dimensional scale and morphology of the surrounding silica nanostructure. We expect that CDA conducted with lipid mixtures (and optionally cholesterol and proteins) will enhance cell viability and allow selective lipid partitioning at the cellular interface, raft formation, and the localization of non-native transmembrane proteins. This in turn should influence membrane fluidity, chemical gradient development, transport of molecules and signals, and membrane healing. Additionally we expect to be able to use the nanostructured host as a reservoir for nutrients and growth factors to control metabolic activity. Finally through control of the pore size, surface chemistry, and connectivity of the nanostructured host we expect to engineer transport characteristics important to cell-cell signaling.

In order to understand CDA and explore its ability to develop more complex and functional interfaces and matrices (mimicking on some level the ECM), we have employed a series of water soluble *di*C_6 lipids as structure directing agents and have monitored the evolving structure with fluorescence microscopy and GISAXS. Figure 1, shows confocal images of yeast immobilized within silica matrices where we included 1% of the corresponding optically labeled lipid analog. We observe that different lipid headgroups result in quite different bio/nano interfaces. Most dramatically switching from phosphotidyl choline (PC) to phosphotidyl ethanolamine (PE) results in an interface where there is no apparent preferential lipid localization. In that the pK_a of these headgroups is similar within half a unit, these results indicate that the cell/lipid interaction (and resulting interface) cannot be explained strictly by electrostatics. GISAXS studies

of this system showed that the PE headgroup also did not 'switch' the nanostructure from 2D hexagonal to lamellar as observed for PC (see CJB *et al. Science* 2006). To fundamentally understand the lipid headgroup/cellular interaction, we are initiating laser tweezer and AFM studies of the effective interaction potential between cells and model supported lipid bilayer systems, lipid bilayer coated beads or supported lipid bilayers, respectively.

To introduce other non-soluble, biologically relevant lipids for CDA, we have prepared water-soluble liposomes that were then introduced during the standard CDA process (with or without additional transmembrane proteins). Figure 2 shows confocal slices of a system prepared with 1% NBD-labeled *di*C_6PC plus POPC liposomes optically labeled with 1% of Texas red labeled DHPE. We observe that both the short chain *di*C_6PC structure directing lipid and the longer chained POPC lipids are localized at the cellular surface. The merged image however suggests that the longer chain lipid is preferentially localized at the cellular interface as well as the interface with the surrounding silica nanostructure (black in image).

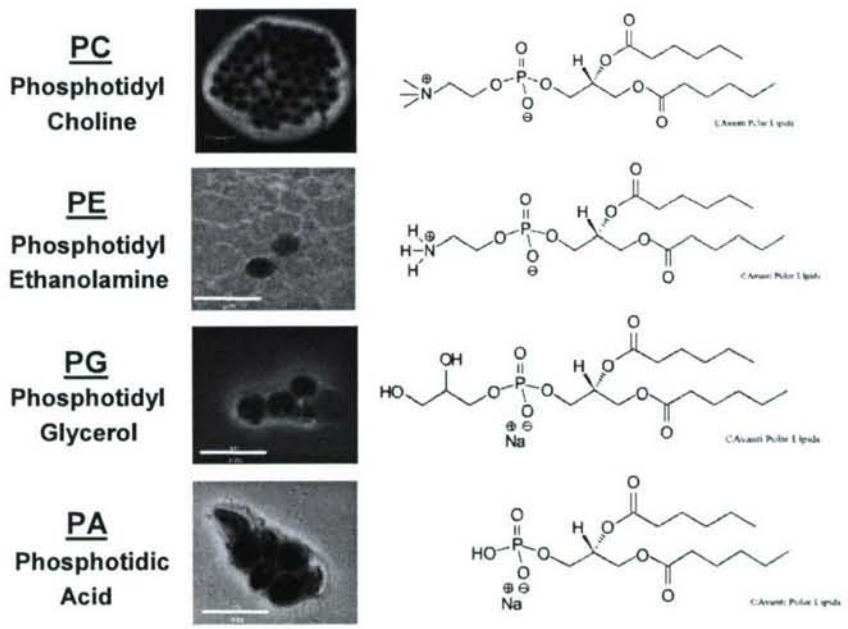

Figure 1. Flourescence confocal images of optically labeled C_6 lipids used in cell directed assembly. Different lipid headgroups result in vastly different extents of lipid localization at the cellular interface (yeast cells appear black).

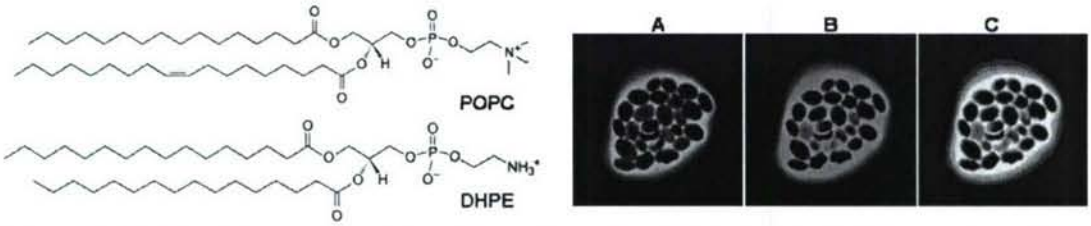

Figure 2. Confocal fluorescence slices of yeast immobilized in *di*C6PC (labeled green) templated silica matrices prepared using CDA with added POPC liposomes. Liposomes included 1% Texas red labeled DHPE, which presumably tracks the location of POPC. Red rims located at the cellular surfaces and at the interface with the surrounding silica matrix (black) suggest preferential lipid localization.

4.1.2. Creating new interfaces by incorporating non-native functional proteins to yield new functionality

Attempts to physically modify the cell surface and introduce new functionality through the integration of non-native proteins have seldom been successful due to the complexity associated with proteins and cells. Therefore, cell modification is usually accomplished via labor intensive, time consuming and expensive genetic modifications. Genetic engineering techniques require extensive specialization, and although considerable progress has been made in genomics and proteomics, there are still numerous physical barriers to overcome when attempting to introduce foreign functionality across cell species and kingdoms. During the past year, we have demonstrated a novel technique that utilizes our CDA process to physically introduce functional bacterial proteins at the surface of yeast cells. Bacteriorhodopsin (BR) is a transmembrane photochromic protein isolated from the purple membrane of the salt marsh halophile, *Halobacterium salinarum*. It acts as an energy transducer, absorbing and converting light into chemical energy. We have introduced BR in to our CDA systems using two approaches: addition of the purified protein directly or incorporation of the BR into a DMPC liposome. Figure 3A-C show that adding the protein directly results in BR localization in a somewhat diffuse region that corresponds closely to the region of *di*C_6PC lipid localization. Figure 3D-F show that introduction of BR in a liposome results in a more conformal region of BR localization and that the longer chain DMPC (labeled green in these panels) preferentially localizes at the cellular surface (consistent with Figure 2). Because BR (and more generally transmembrane protein) functionality requires incorporation in a lipid bilayer with the structure/dimension needed to accommodate the hydrophilic and phobic domains, we hypothesized that, to function as a protein pump, BR would have to be incorporated in a longer chain lipid bilayer. Figure 4 shows pH gradient development for these two approaches. We find that BR introduced in a liposome dramatically changes the pH gradient. This suggests the BR is functional and preferentially oriented at the cellular surface. These results point out a completely new approach in which proteins with unusual properties can be isolated from one organism and physically introduced at the surface of another organism to provide new non-native functionalities.

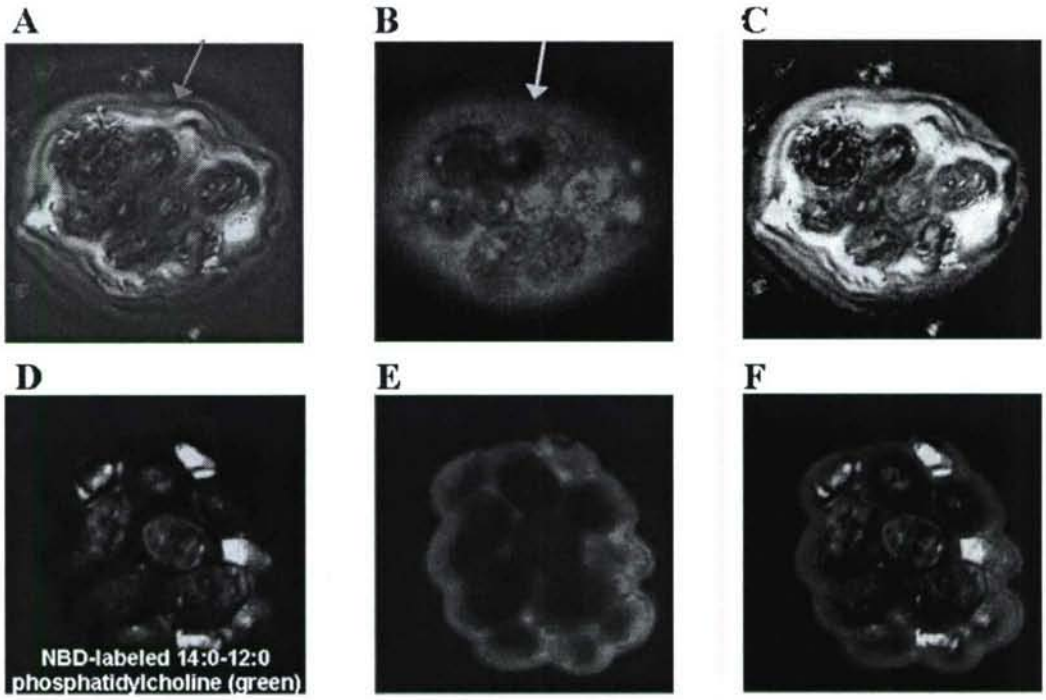

Figure 3. Confocal fluorescence slices of red emission fluorophore labeled Bacteriorhodopsin (BR) localized around yeast cells using cell-directed assembly. (A-C) BR added directly during CDA where the green label is on the $di C_6PC$ structure directing agent. (D-F) BR added from liposome. (D) fluorescently labeled POPC lipid (green). (E) Fluorescently labeled Bacteriorhodopsin (red) and (F) a merged image showing co-localization of the lipid and protein around the cells. These three panels show that POPC is preferentially localized at the cell surface. BR incorporation in POPC should allow it to achieve its native functionality.

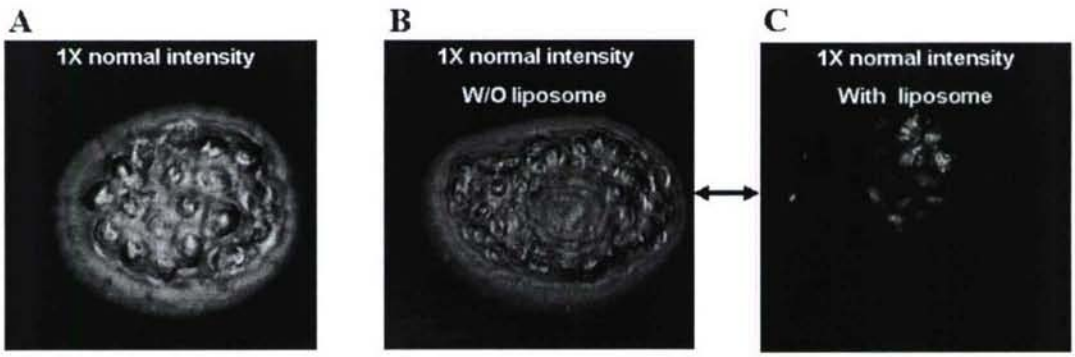

Figure 4. Comparison of pH gradient development with: (A) yeast prepared by CDA, (B) BR added directly during CDA, (C) BR introduced in liposome. The labeled dye is pH sensitive and lower pH results in weaker fluorescence.

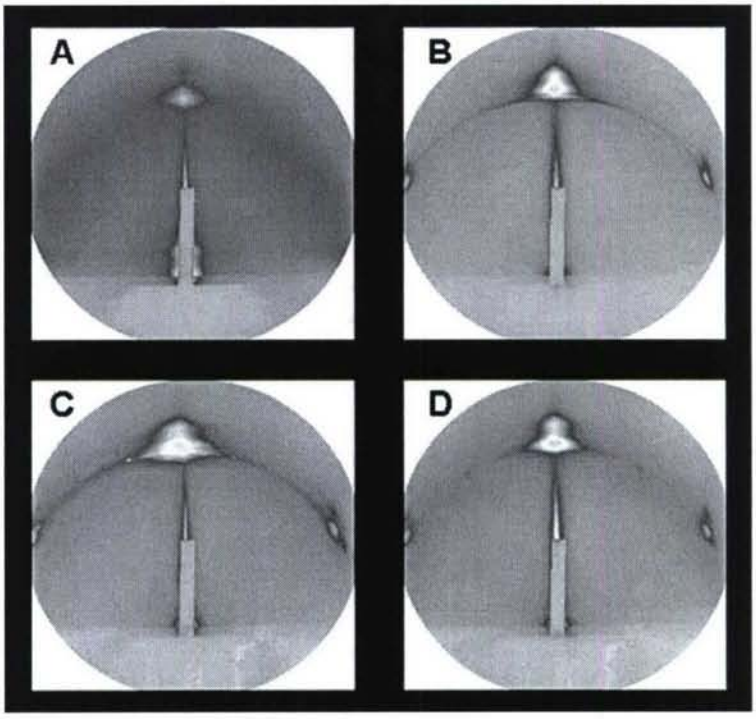

Figure 5. GISAXS patterns of various cell types immobilized using CDA: (A) *S. cerevisiae*, (B) *E. coli*, (C) *B. subtilis*, (D) *T. aquaticus*. Patterns B-D represent a 2D hexagonal mesophase. Pattern A is that of a lamellar mesophase. Finer scale details of the 2D spot patterns are presumably associated with the detailed structure of the bio/nano interface.

4.2. Extend cell-directed assembly to immobilize various cell types

4.2.1. Extension to various microorganisms

To extend the applicability of our CDA process, we have continued to explore the structure and properties of systems prepared with new organisms and cells - model organisms which provide a baseline to understand the influence of nanostructuring on cellular behavior, unique organisms which may allow the development of unprecedented functions in cell-based devices, and mammalian cells. For example, we have demonstrated CDA with a model eukaryote, *S. cerevisiae*, a model gram-negative bacterium, *E. coli*, and a model gram-positive bacterium *S. epidermidis*. We have also investigated CDA with the anthrax analogue *B. subtilis* for potential biodefense studies, the tuberculosis analogue *M. smegmatis* for potential infectious disease studies, as well as extremophiles like *T. auquaticus*, which lives in high-temperature environments, and *D. radiodurans*, which withstands extraordinary exposures to DNA damaging radiation. We find that all these microorganisms can be successfully immobilized

via CDA. However each organism or cell displays its own unique behavior/interface. We have studied these interactions using grazing-incidence small-angle X-ray scattering at the Advanced Photon Source at Argonne National Laboratories (Figure 5). We find that each cell type has a unique ability to localize the lipid surfactant and establish local pH and ion gradients that in turn effect the development of the silica nanostructure probed by GISAXS. In particular extensive lipid localization at the yeast surface is correlated with the switching of the 2D hexagonal mesophase to a lamellar mesophase, which is not observed for organisms which exhibit lower extents of lipid localization.

4.2.2 Extension to model mammalian cells

Our previous research has demonstrated CDA as a general immobilization route for various single-celled organisms. To enhance the viability of immobilized cells, we have investigated new media in which to conduct CDA that incorporate essential nutrients and allow the nanostructured silica host to serve as a nutrient reservoir. In this fashion we maintain cellular access to necessary resources and avoid the requirement for constant regeneration of nutrients common to all other cell immobilization techniques to date. Supply of nutrients is especially important for extending CDA to mammalian cell lines. Whereas single-celled organisms can senesce, or pause their metabolic activity until more nutrients are available, mammalian cells quickly expire without constant access to nutrients and other chemical factors. We expect immobilization of mammalian cells to be important for tissue engineering, directing cell differentiation, and studying the onset of disease.

We have used CDA to incorporate model mammalian cells, such as adherent human embryonic kidney (HEK) cells, into our lipid-templated host nanostructures and have found that mammalian cells localize and internalize fluorescently-labeled phospholipids in a fashion similar to, yet distinct from, single-celled eukaryotes and prokaryotes (Figure 6A). To assess the viability of the HEK cells immobilized via CDA, fluorescent probes were utilized. Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to the fluorescent calcein, producing an intense uniform green fluorescence in live cells (Figure 6 A and B). Ethidium homodimer enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (Figure 6C). With the usual CDA protocol, we determined that the number of cells alive after one hour is at least equal to the number of cells alive in buffer, indicating that the CDA immobilization process does not cause irreversible damage to the cell. We will further investigate the ability to introduce minimal essential media during the immobilization process to extend the viability and functionality of mammalian cells immobilized using cell-directed assembly, allowing us to investigate many cellular processes including the onset of various diseases.

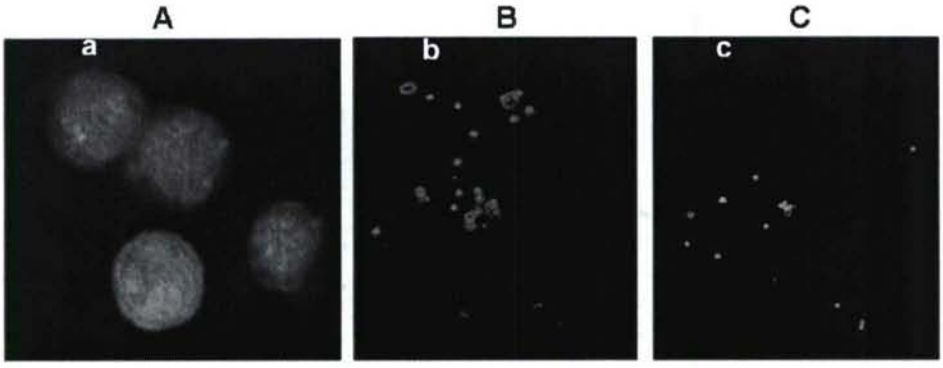

Figure 6. (A) HEK cells immobilized in a nanostructured silica film via CDA with fluorescent-green phospholipid. Cells are stained with a red fluorescent probe. (B) and (C) fluorescent images showing the viability of immobilized HEK cells with live cells appearing green and dead cells appearing red. Images are of the same field with colors separated for clarity in assessment.

4.3 Patterning of cells to create functional multi-cellular materials with engineered cellular communication

4.3.1 Cellular Integration into pre-assembled lipid/silica mesophase films

This year we have confirmed our discovery of cellular integration into pre-formed lipid/silica meosphase films (Figure 7) and have extended this approach to bacterial and mammalian cell lines. Remarkably cells introduced in water, buffer, or media onto an ordered lipid- or glycerol monoleate templated surface cause almost immediate rearrangement of the lipid/silica matrix to create a bio/nano interface quite similar to that formed by direct CDA. This approach has several advantages over CDA: 1) as drying and self-assembly of the lipid/silica mesophase film occurs prior to introduction of cells, the cells are not exposed to solvent and acid catalyst, and we expect any associated osmotic stress to be reduced. 2) this approach preserves the original nanoscale architecture directed by the chosen lipid template. 3) The approach is amenable to patterning using direct write procedures like ink-jet printing or selective wetting (see more in next section), which should allow us to pattern cellular arrays with differing cell densities and connectivities. Figure 7A below shows a schematic of the approach. Confocal imaging of the green labeled lipid introduced in the pre-made film indicates that after cellular integration the lipid envelops the cell (Figure 7B). Corresponding viability studies indicate that integrated yeast and bacterial cell lines have comparable viability as those introduced by direct CDA. The most fascinating discovery in this area is the preliminary result shown in Figure 7C of a mammalian (mouse) rat macrophage cell which has apparently integrated itself into a pre-made *di*C_6PC/silica mesophase film. This structure observed in SEM without fixation is stable upon evacuation and electron beam exposure.

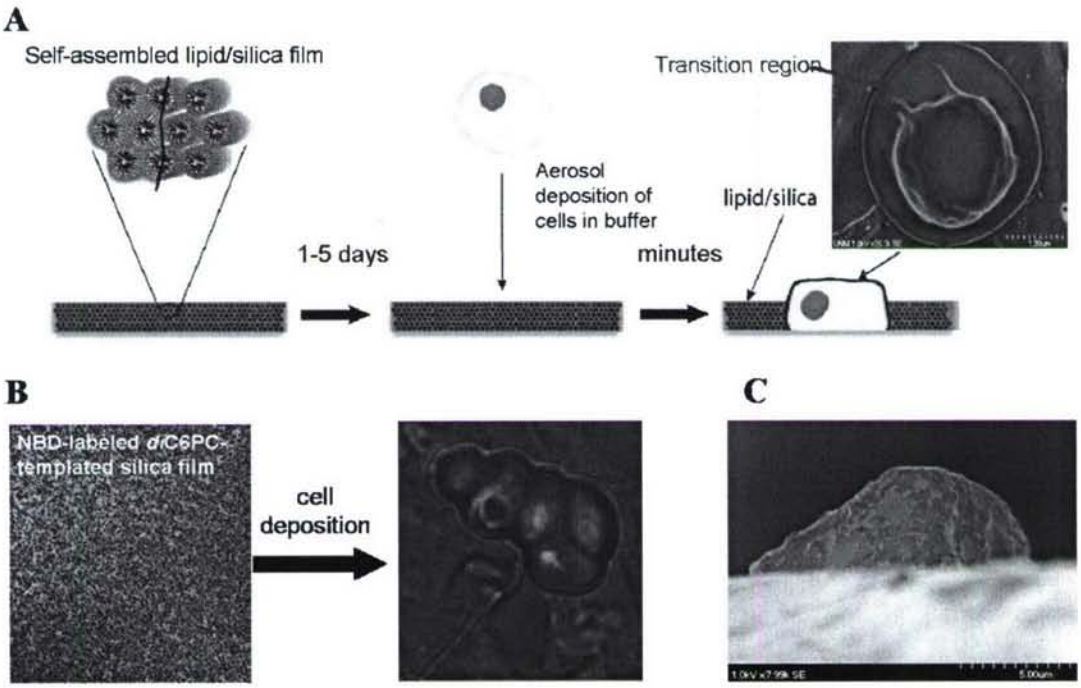

Figure 7. Cellular integration into pre-made diC$_6$PC/silica mesophase film. A) Schematic of process and SEM image of immobilized yeast cell, B) confocal microscopy of green labeled diC$_6$PC/silica pre-made film (left) and slice near top of integrated cell (right). C) SEM cross-sectional view of integrated mouse macrophage cell.

4.3.2 Selective wetting to pattern living cells into functional and connected arrays

In order to control the placement of cells and their spacio-temporal interactions, we have developed a novel optical patterning technique, which is not only simple and direct but also biologically compatible. Nanostructured silica films are created via evaporation induced self-assembly of aqueous silica precursors with a biologically compatible surfactant, glycerol monooleate (GMO) via dip-coating, spin-coating, drop-casting, or aerosol deposition. GISAXS studies show the films to form a highly ordered cubic mesostructure. The surfactant can be removed by calcinations or by UV/ozone lithography to yield a porous silica film.

To create patterned regions for cellular integration into the nanostructured silica matrix, we exploit the change in the hydrophobicity and fluidity of the surface of the film upon UV/ozone exposure. When the film is first deposited, it has a relatively low contact angle with water and remains in a semi-solid state. Upon exposure to UV/ozone, the GMO begins to photodecompose and the silanol precursors become more condensed. This yields a pattern where UV/ozone exposed regions are more hydrophobic and solidified and adjoining unexposed regions are more fluid and hydrophilic. Longer exposure to UV/ ozone removes the surfactant entirely, leaving a porous and extremely hydrophilic film surface.

This trend is shown below in Figure 8A. The use of a standard UV lithographic mask on top of the film allows for the definition of hydrophobic/hydrophilic regions when exposed to UV/ozone. The areas that are blocked by the mask remain hydrophilic, while exposed areas are more hydrophobic. Once these regions are defined, living cells introduced in water (or a water-based nutrient media) selectively localize to the defined hydrophilic regions. We have demonstrated this optically-controlled patterning method with many types of cells. Figure 8B shows an example with yeast cells, where the yeast is localized on the unexposed regions.

The functionality of this system has also been further extended using the ability to remove the surfactant to regain a hydrophilic film that is now porous. After deposition of the cells onto the hydrophilic/hydrophobic patterned film, the degraded surfactant in the hydrophobic portions can be completely removed using UV light and ozone, leaving porous, hydrophilic portions of the film. With the appropriate pattern on a UV mask, this technique can be used to create porous regions between the localized cells that can be used to introduce nutrient media, growth factors, toxins, or other molecules of interest, as shown below in Figure 8C. These techniques, when used in conjunction, form a new, simple yet powerful way to integrate traditional lithography with living cells.

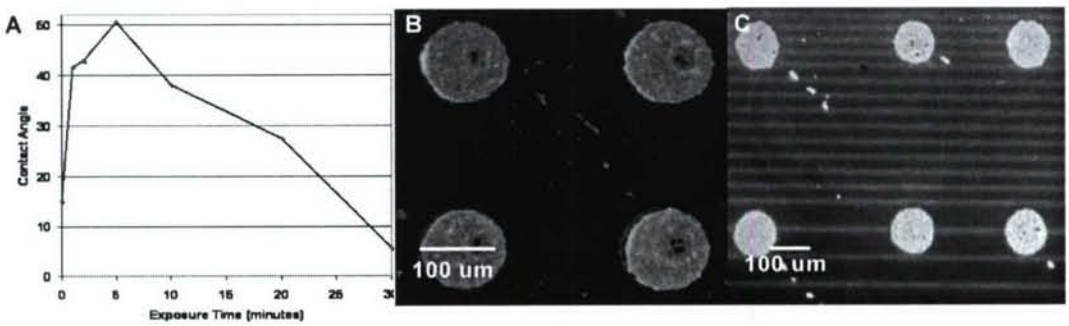

Figure 8. A) Water contact angle of a GMO-templated silica film as a function of UV light and ozone exposure time, B) Localization of fluorescently-tagged yeast onto the hydrophilic regions of an optically patterned nanostructured silica film, and C) Porous regions in connecting patches of cells have been created through further UV/ozone exposure and the resulting regions have been filled with a fluorescent nutrient media for visualization.

4.3.2 A potentially new pathway of cellular communication among individual nanoconfined cells

In a first step toward establishing a platform for studying cell-cell communications, we have investigated the influence of the silica nanostructure on the concentration gradients of signaling molecules of immobilized cells. We have immobilized cells in picoliter-sized nanostructured droplets to ascertain the ability of cells to quorum sense over a much smaller concentration than is

generally observed. Using genetically modified S. aureus that express green-fluorescent protein when they reach their quorum limit which have a normal quorum threshold observed at ~10^9 cells/mL, we have observed two cells quorum sensing in a 2 pL droplet (Figure 9). The effective concentration of cells in this droplet exceeds the quorum limit, leading to the observed response in a minimal number of cells and revealing that the nanostructure can be used to influence the concentration gradient of sensing molecules. In future work we will seek to utilize this newfound understanding of individual cell communication, along with our expertise in a variety of biocompatible cell patterning techniques, to study the communication of cells spanning the quorum limit.

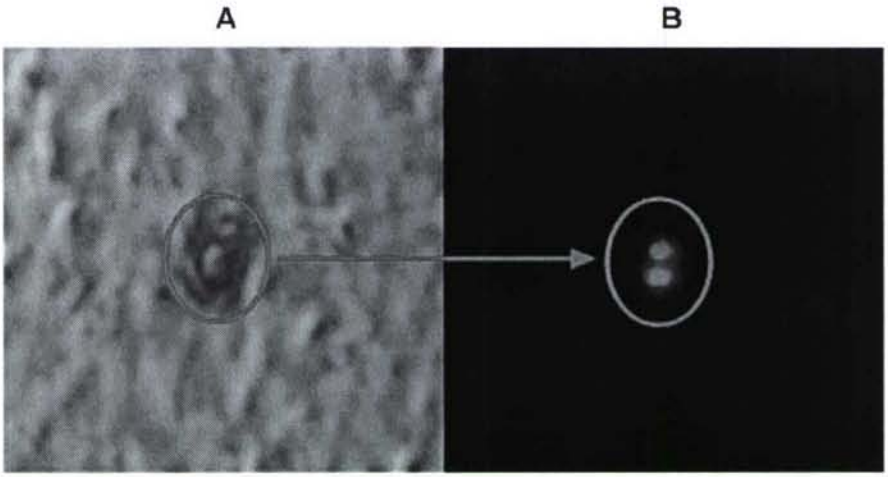

Figure 9. Quorum sensing of cells at concentrations well under the quorum threshold: (A) phase-contrast image of 2 Staphylococcus aureus cells encapsulated in a 2 picoliter nanostructured silica droplet, (B) corresponding fluorescence image showing active quorum sensing by the cells as indicated by the expression of GFP.

5. Personnel Supported

Faculty

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Undergraduates

DeAnna Lopez

Cynthia Douthit
Shelly Karlin
Jennifer Pelowitz
Landon White

6. Publications (from August, 2006 to August, 2007)

Publications in Refereed Journals:

1. **Modulus–density scaling behaviour and framework architecture of nanoporous self-assembled silicas,** Hongyou Fan, Christopher Hartshorn, Thomas Buchheit, David Tallant, Roger Assink, Regina Simpson, Dave J. Kissel, Daniel J. Lacks, Salvatore Torquato, and C. Jeffrey Brinker, Nature Materials, June 2007, 6, p. 418-423.

2. **Optical Detection of Ion-Channel-Induced Proton Transport in Supported Phospholipid Bilayers,** Tsung-Han (Calvin) Yang, Chanel K. Yee, Meri L. Amweg, Seema Singh, Eric L. Kendall, Andrew M. Dattelbaum, Andrew P. Shreve, C. Jeffrey Brinker, and Atul N. Parikh. Nano Letters.; **2007;** ASAP Web Release Date: 13-Jul-2007.

3. **Cell-Directed Assembly of Bio/Nano Interfaces—A New Scheme for Cell Immobilization,** Helen K. Baca, Eric Carnes, Seema Singh, Carlee Ashley, Deanna Lopez, and C. Jeffrey Brinker, Acc. Chem. Res.; **2007;** ASAP Web Release Date: 03-Aug-2007.

4. **Amphotericin B channels in phospholipid membrane-coated nanoporous silicon surfaces: Implications for photovoltaic driving of ions across membranes,**Yilma, Solomon; Liu, Nangou; Samoylov, Alexander; Lo, Ting; Brinker, C. Jeffrey; Vodyanoy, Vitaly. Biosensors and Bioelectronics, Mar 15 2007, **22**, no.8, p.1605-1611

5. **Nanocrystalline mesoporous palladium activated tin oxide thin films as room-temperature hydrogen gas sensors,** De, Goutam; KÖhn, Ralf, Xomeritakis, George; and Brinker, C, Jeffrey, Chemical Communications, published on the web February 12, 2007.

6. **Large-conductance cholesterol-amphotericin B channels in reconstituted lipid bilayers**, Yilma, S; Cannon-Sykora, J; Samoylov, A; Lo, T; Liu, N; Brinker, CJ; Neely, WC; Vodyanoy, VBiosensors and Bioelectronics, Feb 15 2007, **22,** no.7, p.1359-1367

7. **Anodic alumina supported dual-layer microporous silica membranes** , Xomeritakis, G; Liu, NG; Chen, Z; Jiang, YB; Kohn, R; Johnson, PE; Tsai, CY; Shah, PB; Khalil, S; Singh, S; Brinker, CJ. Journal of Membrane Science, Jan 15 2007, **287,** p.157-161.

8. **Two-photon absorption of matrix-free Ge nanocrystals,** Gerung, H; Zhao, Y; Wang, L; Jain, RK; Boyle, TJ; Brinker, CJ; Han, SM, Applied Physics Letters, Sept 11, 2006, **89** p. 111107, 1-3

9. **Nanometer-thick conformal pore-sealing of self-assembled mesoporous silica by plasma-assisted atomic layer deposition,** Jiang, YB; Liu, NG; Gerung, H; Cecchi, JL; Brinker, CJ.Journal of The American Chemical Society, August 30, 2006, **128**, p. 11018-11019.

10. **Drying transition of confined water**
Singh, S; Houston, J; van Swol, F; Brinker, CJ.
Nature, Aug 3 2006, **442**, p. 526.

11. **Drag reduction on a patterned superhydrophobic surface**
Truesdell, R; Mammoli, A.; Vorobieff, P; van Swol, F; Brinker, CJ.
Physical Review Letters, July 28, 2006, **97**, p. 044504.

12. **Nanoporous carbon nanotubes synthesized through confined hydrogen-bonding self-assembly**
Rodriguez, AT; Chen, M; Chen, Z; Brinker, CJ; Fan, HY
Journal of the American Chemical Society, July 26 2006, **128**, p. 9276-9277

13. **Cell-directed assembly of lipid-silica nanostructures providing extended cell viability**
Baca, HK; Ashley, C; Carnes, E; Lopez, D; Flemming, J; Dunphy, D; Singh, S; Chen, Z; Liu, N; Fan, HY, Lopez, GP; Brozik, SM; Werner-Washburne, M; Brinker, CJ
Science, July 2006, **313**, p. 337-341

Papers / Chapters Submitted

1. **Directed aerosol writing of ordered silica nanostructures on arbitrary surfaces with self-assembling inks**
Pang, J; Stuecker, JN; Jiang, YB; Bhakta, AJ; Li, Peng; Cesarano, J; Sutton, D; Calvert, P; and Brinker, CJ. SMALL, in review

2. **Photoresponsive Nanocomposite Materials Including Axobenzene-Containing Polysilsesquixane Films and Photoswitched Nanovalves**
Nanguo Liu and C. Jeffrey Brinker
in **Smart Light-Responsive Materials: Azobenzene Containing Polymers and Liquid Crystals,** Yue Zhao and Tomiki Ikeda, eds., John Wiley & Sons, Inc., Hoboken, NJ (2007)

Books and Book Chapters

1. **Annual Review of Nano Research – Volume 1**
Cao, Guozhong and Brinker, C. Jeffrey, editors
World Scientific Publishing Co. Ltd., Singapore/London, 2006

Proceedings Papers

1. **Characterization of superhydrophobic materials using multiresonance acoustic shear wave sensors**
Kwoun, SJ; Lec, RM; Cairncross, RA; Shah, P; Brinker, CJ
IEEE Transactions on Ultrasonics, Ferroelectrics and Frequency Control; Aug. 2006; vol.53, no.8, p.1400-3

2. **A novel design toward understanding and characterizing transport behavior of composite mesoporous silica thin films**
   Chen, Z; Adams, DP; Vasile, MJ; Liu, NG; Jiang, YB; Xomeritakis, G; Brinker, CJ
   Materials Research Society Proceedings, vol 921, 2006.

7. Interactions Transitions

Impact and Publicity

Brinker work recognized through high impact publications, citations, awards, and media coverage:

- Seminal *Nature* paper on Evaporation-Induced Self-Assembly (CJB corresponding author) recognized by ISI as Top 20 Paper of Decade (paper cited 100 more times since announcement)
- Named University of New Mexico Regent's Professor
- Citation H-index increased from 40 to 44 (44 papers cited 44 or more times) – CJB corresponding author on all 44 papers.
- Appointed: Professor of Molecular Genetics and Microbiology, UNM
- Press Coverage of Cell Directed Assembly and Superhydrophobicity:
  - *C&E News,*
  - *Nature Chemical Biology,*
  - *Scientific American,*
  - *Nanomaterials News,*
  - *Press Conference American Physical Society Annual Meeting,*
  - *Interview: German Public Radio, many other*

Invited Talks at Meetings, Conferences, Seminars, etc.

1. September, 2007, Invited Tutorial: *Fundamentals of Sol-Gel Technology, Sol-Gel 2007,* Montpellier France
2. September 1, 2007, *Cell Directed Assembly and its Extension to Lithography with Life, Sol-Gel,* Montpellier, France
3. SNL LDRD Day - *Superhydrophobic Surfaces for Microfluidics and MEMS*
4. *Chemistry and Physics of Liquids Gordon Conference* – experiments on superhydrophobic surfaces (*Nature*, 2006)
5. August 14, 2007, International Conference on BioNano Materials ICBN, *Directing Inorganic Self-Assembly with Living Cells,* Biopolois, Singapore.

6. June 1, 2007. Department of Chemical and Environmental Engineering, University of California – Riverside, *Evaporation Induced Self-Assembly and its Extension to Living Cell-Directed Assembly of Novel Bio/Nano Interfaces,* Riverside, CA.

7. May 29, 2007. Argonne National Laboratory, *Directing the Assembly of Nanostructured Films with Living Cells*, Argonne, IL.

8. March 30, 2007. Cabot Corporation, *Evaporation-Induced Self Assembly (EISA) of Porous and Composite Nanostructures,* Billerica, MA

9. March 5-9, 2007. Spring 2007 Meeting of the American Physical Society, *Directing the Assembly of Nanostructructured Films with Living Cells,* Denver, CO.

10. October 26, 2006. Macromolecular Science and Engineering Symposium, University of Michigan, *Evaporation-Induced Self-Assembly of Porous and Composite Nanostructures*, Ann Arbor, MI.

11. October 11-12, 2006. 2006 International Institute for Nanotechnology Symposium, Northwestern University, *Evaporation Induced Self-Assembly of Functional Nanostructures*, Evanston, IL.

12. September 19, 2006. Argonne National Laboratory, Dedication of Argonne's Center for Nanoscale Materials, *Evaporation-Induced Self-Assembly of Porous and Composite Thin Film Nanostructures*, Argonne, IL.

13. September 18, 2006, Northwestern University, Materials Science Department, *Symbiotic Assembly of Bio/Nano Interfaces and Architectures*, Evanston, IL.

14. September 10-14, 2006. 232nd American Chemical Society National Meeting & Exposition, *Bridging the Gap: Observation of Ultra Long Range Hydrophobic Interactions,* San Francisco, CA.

Contributed Presentations

1. Carnes, EC ; Ashley, CE ; Lopez, DM ; Douthit, CM ; Singh, S ; Dunphy, DR ; Brinker, CJ. *Integration of Living Cells within Self-Assembled Nanostructures,* 43rd Annual Symposium New Mexico Chapter of the American Vacuum Society, May 21-22, 2007, Albuquerque, NM. (undergraduate student poster competition 1st prize)

2. Ashley, CE ; Carnes, EC ; White, L ; Yuan, Zhen ; Dunphy, DR ; Petsev, D ; Atanassov, P ; Peabody, D ; Wang, J ; Brinker, CJ. *Grazing Incidence Small Angle X-Ray Scattering (GISAXS) Characterization of 2D Bacteriophage Arrays Deposited via Convective Assembly,* 43rd Annual Symposium New Mexico Chapter of the American Vacuum Society, May 21-22, 2007, Albuquerque, NM. (graduate student paper competition 1st prize)

3. Carnes, EC ; Ashley, CE ; Lopez, DM ; Baca, H ; Singh, S ; Dunphy, DR ; Brinker, CJ. *Cell-Directed Assembly : A New Means of Bio/Nano Integration,* Center for Integrated Nanotechnologies (CINT) Annual Program Review, April 18-20, 2007, Sandia National Laboratories, Albuquerque, NM.

4. Jiang, YB; Xomeritakis, G; Chen, Z; Dunphy, D; Pang, J; Branson, ED; Cecchi, JL; Brinker, CJ. *Remote Plasma Assisted Atomic Layer Deposition of Ultra-Thin Pore-Sealants for Self-Assembled Porous Low-k Materials*, Materials Research Society Spring Meeting, Symposium B: Materials, Processes, Integration and Reliability in Advanced Interconnects

for Micro- and Nano-Electronics, April 9-13, 2007, San Francisco, CA.

5. Carnes, EC ; Ashley, CE ; Lopez, DM ; Douthid, CM ; Karlin, SA ; Pelowitz, J ; Gresham, H ; Timmins, G ; Brinker, CJ. Patternable Integration of Living Cells with Self-Assembled Nanomaterials, Materials Research Society Spring Meeting, Symposium N: Printing Methods for Electronics, Photonics, and Biomaterials, April 9-13, 2007, San Francisco, CA.

6. Ashley, CE ; Carnes, EC ; Chen, L ; Yuan, Z ; Jiang, YB ; Calderia, J ; Petsev, D ; Atanassov, P ; Peabody, D ; Brinker, CJ. *Co-Assembly of Genetically-Modified Viruses and Metal Nanoparticles into 3D Arrays via a Novel Deposition Technique*, Materials Research Society Spring Meeting, Symposium S: Synthesis, Processing and Properties of Organic/Inorganic Hybrid Materials, April 9-13, 2007, San Francisco, CA.

7. Pang, J; Stuecker, JN; Bhakta, A; Jiang, YB; Cesarano, J; Calvert, P; Sutton, D; Brinker, CJ. *Aerosol-Robo-Printing : Writing Ordered Nanostructures on Arbitrary Surfaces with Self-Assembling Inks*, Materials Research Society Spring Meeting, Symposium D : Deposition on Non-Planar Substrates, April 9-13, 2007, San Francisco, CA.

8. Jiang, YB ; Chen, Z ; Brinker, CJ. *Sub-Angstrom Tuning of Channel Pore Size and Cell Surface Replication with Atomic Layer Deposition and Plasma-Assisted ALD*, Annual Meeting of the NIH National Center for Design of Biomimietic Nanoconductors, February 28 - March 2, 2007, Bethesda, MD.

9. Jiang, XM ; Fan, HY ; Singh, S ; Brinker, CJ. *Construction of Cell Mimetic Architectures for Probing Inter-Extra-Cellular Communication,* Annual Meeting of the NIH National Center for Design of Biomimietic Nanoconductors, February 28 - March 2, 2007, Bethesda, MD.

10. Yang, TH ; Yee, CK ; Amweg, ML ; Dattlebaum, AM ; Shreve, AP ; Clarke, J ; Bayley, H ; Singh, S ; Brinker, CJ ; Parikh, AN. *New Hybrid Bilayer Constructs Enabling Optical Definition of Protein Location and Function,* Annual Meeting of the NIH National Center for Design of Biomimietic Nanoconductors, February 28 - March 2, 2007, Bethesda, MD.

11. Brinker, CJ. Self-Assembly of Bio-Inspired and Bio-Directed Nanostructures. *2007 AFOSR Biomimetic, Biomaterials and Biointerfacial Sciences Program Review*, Duck Key, FL, January 8-12, 2007.

12. Carnes, E., Ashley, C., Lopez, D., Douthit, C., Karlin, S., Pelowitz, J., Wise, A., Singh, S., Brinker, C.J., *Creation of Integrated Platforms for Engineered Cell-Cell Communication via Cell Directed Assembly*, Materials Research Society Fall 2006 Meeting, November 27-December 1, 2006, Boston, MA.

13. Brinker, C.J., Carnes, E., Ashley, C., Singh, S., *Engineered Bio/Nano Interfaces via Cell-directed Assembly*, Materials Research Society Fall 2006 Meeting, November 27-December 1, 2006, Boston, MA.

14. Jiang, Y.B., Cecchi, J.L., Brinker, C.J., *Plasma Controlled Atomic Layer Deposition for Sealing Pores in Low-k Materials*, American Vacuum Society 53rd International Symposium, Plasma Science and Technology Session, November 12-17, 2006, San Francisco, CA

15. Douthit, C.M., Carnes, E.C., Ashley, C.E., Lopez, D., Pelowitz, J., Karlin, S.A., Brinker, L.M., and Brinker, C.J., *In-Situ Genetic Modification through Cell-Directed Assembly,* 18th

Annual Rio Grande Symposium on Advanced Materials – RGSAM, October 10, 2006, Albuquerque, NM.

16. Johnson, P., Branson, E.D., Singh, S., and Brinker, C.J., *Gecko Inspired Super Adhesives,* 18th Annual Rio Grande Symposium on Advanced Materials – RGSAM, October 10, 2006, Albuquerque, NM.

Transitions – Professional Communications

- January 2004 – present
  Stuart Burchill, CEO
  Industrial NanoTech, Inc.
  Corporate Office
  109 East 17th Street, Suite 15
  Cheyenne, WY 82001
  800-767-3998

This collaboration involves the development of new coatings for specialty insulation applications in high efficiency materials for architectural and manufacturing use. Mesoporous or aerogel materials tailored for specific applications, e.g. UV resistance, water repellancy, high R-value, will replace traditional ceramic particle fillers.

- June 2004 – present
  Daniel Roitman
  Agilent Laboratories
  3500 Deer Creek Road
  Palo Alto, CA 94303
  650-485-5958

Exploration of the use of mesoporous films for immobilization of ligands for biomolecular sensing applications using surface plasmon resonance.

Transitions – Professional Collaborations

• **Customer:** UniPixel, The Woodlands, Texas 77381, Phone (281) 825-4500

Results: We have entered into a multiyear effort with Uni-Pixel to build prototype displays based on a UPD disruptive display concept that will utilize our ultra-low density/low refractive index coatings. We are also partnering with Lockheed-Martin Corp to build a prototype to demonstrate this technology for military and aerospace applications.

Applications: Transparent, ultra-thin, high-resolution flat panel displays. Aircraft canopies, automobile windhields, information & status displays, entertainment devices, surface enhanced Raman spectroscopy platforms.

• **Customer:** David Sutton, Paul Ferm, ICI/National Starch, +44 (0)1642 435857, 908-685-5323, paul.ferm@nstarch.com

Results: ICI/National Starch initiated a contracted with our group to transition our evaporation induced self-assembly process (*Nature*, 1997 etc.) to direct writing of nanocomposite architectures using ink-jet and aerosol printing approaches. We have demonstrated direct writing of nanoporous materials and are now extending this work to nanocomposite (silica/polymer) materials (*Nature,* 1998). We have also interested National Starch is also interested in using our aerosol assisted self-assembly approach (*nature*, 1999) to create nanoporous particles for controlled release of fragrances. This controlled release problem may also benefit from the use of a photo-trigger to initiate the release. For this we are considering our azobenzene approach to make nano-valves (*Nano Lett.,* 2004).

Applications: direct write flexible electronics, controlled release nanostructures

• **Customer:** General Motors Corp Fuel Cell Activities, Rick Blunk, Tel-585-624-6600

Results: Multicomponent Silicate Hydrophilic Coatings for Metal Bipolar Plates - General Motors Fuel Cell Activities (FCA) is seeking a stable hydrophilic coating for its stainless steel (SS) bipolar plates. Plate hydrophilicity is required to meet FCA's low power stability (LPS) targets. A hydrophilic plate surface enables fuel cell-generated water droplets to imbibe into the reactant gas flow field channels (anode and cathode), forming a thin water film on the bottom of the channels. Reactant gases can then pass readily over the film (low transport resistance) and flow successfully to the catalyst layers for 100% electrode utilization, where they participate in the oxidation and reduction chemical reactions. We are developing for GM stable, hydrophilic and potentially superhydrophilic coatings based on both evaporation induced self-assembly of bridged silsesquioxane precursors (*JACS*, 2000) into mesoporous films and traditional sol-gel coating methods to achieve microporous films.

• **Customer:** Stuart Burchill, Industrial NanoTech, Inc., 109 East 17th Street, Suite 15, Cheyenne, WY 82001, 800-767-3998

Results: Coatings with 30 vol% aerogel fillers resulted in substantial improvements in insulating properties and excellent coatability. Organic matrix is tailored for compatibility with hydrophobic particles. CRADA expanded 5X in August 2006.

Applications: Highly insulating coatings for architectural and industrial applications, e.g. heating/air conditioning ducts, factory roofing, refrigerated transport units in ships and railcars, industrial storage containers

• **Customer:** Luna Innovations, Contact: Bryan Koene, Ph.D., Phone (540)558-1699, koeneb@lunainnovations.com_

Results: This Phase I/II STTR Program in the area of ultrahydrophobic coatings will result in materials with superior properties for numerous applications. For example the following areas of active interest to Luna will be impacted by this research:

1. Anti Corrosion • Luna has several ongoing programs in the area of corrosion inhibiting coatings. The addition of a hydrophobic surface to these will be a great benefit to this work. If corrosive materials cannot permeate the surface in the first place, it will greatly extend the life of the coating and the underlying surfaces. • This year we used neutron reflectivity to access whether a porous superhydrophobic coating can prevent/impede corrosion of aluminum immersed in salt water. We observed over a 5X reduction in incipient corrosion compared to the bare Al alloy.

2. Chemical Protective Textiles • Luna has an effort in selectively permeable materials for chemical and biological warfare agent (CBWA) defense. The application of the hydrophobic coatings to membrane materials that possess high permeability of water will result in highly effective CBWA protective fabrics. • On the proposed program, Luna will apply ultrahydrophobic coatings to selectively permeable materials and fabrics to evaluate their effect on chemical protection.

8. New Discoveries, Inventions, Patent Disclosures

Patent Disclosure

1. Jiang, Xingmao and **Brinker, C. Jeffrey**
   Highly Active Titanium Oxide Photooxidation Catalyst
   UNM-795, submitted 9/26/2006

2. Jiang, Xingmao and **Brinker, C. Jeffrey**
   Methods for Preparing High Crystallinity and Surface Area Porous Anatase
   submitted 11/7/2006

3. El-Kady, I., Luk, T.S., **Brinker, C. Jeffrey,** Fan, Hongyou, and Subramania, G.S.
   Sample-Uniform Reproducible Surface Enhanced Raman Spectroscopy (SUR-S)
   Sandia National Labs Disclosure of Technical Advance SD#10584/S-111178, submitted 12/18/2006

4. Pang, Jiebin and **Brinker, C. Jeffrey**
   Supported and Unsupported Ultra-Thin Films of Monolayer Nanoparticles
   Sandia National Labs Disclosure of Technical Advance SD#10681, submitted 3/22/2007

5. Jiang, Ying-Bing, Cecchi, J.L. and **Brinker, C. Jeffrey**
   Microporous Membranes Based on Surface Activation and Reaction
   Sandia National Labs Disclosure of Technical Advance SD#10681704, submitted 4/13/2007